

## OPEN PEER REVIEW REPORT 1

**Name of journal:** Neural Regeneration Research

**Manuscript NO:** NRR-D-21-00033

**Title:** ATP-P2X4R signaling mediates Nod-like receptor family protein 3 inflammasome activation in a male rat model of Parkinson's disease

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**Reviewer's country:** USA

### COMMENTS TO AUTHORS

The authors are to be congratulated on a truly exhaustive investigation of the role of purinergic signaling in inflammatory processes that may contribute to degeneration of the dopaminergic neurons of the substantia nigra in Parkinson's disease. The hypotheses are well formulated, the experimental designs are innovative and approach the question using multiple mutually reinforcing techniques. Careful controls are used throughout. This represents a novel and important addition to our understanding of the role and regulation of inflammation in degeneration of the midbrain dopaminergic system as seen in PD.

(1) I would caution the authors not to equate the 6-OHDA rat rotation model with Parkinson's disease. The rat model is just that--a model. PD is a complex set of syndromes that likely represent the final common pathway of more than one process, and it is not entirely clear to which of these, if any, administration of a neurotoxin like 6-OHDA is most similar. The inflammation and cell death caused in this model may not be exactly similar to those of the sporadic or genetic forms of the human disease. It would be helpful to draw this distinction in the introduction and discussion and to refer to the rats as "Parkinsonian" or as an "animal model of PD" as opposed to calling them PD rats or equating the model with the disease.

(2) For similar reasons, I would advise the authors to avoid statements such as "because PD is similar to AD" (p.4 line 33). This statement is overly broad. In what specific way is it similar?

(3) There is evidence suggesting differences between male and female in activation of NLRP3. This is presumably the reason that the authors have specified male rats as the model. This should be mentioned in the introduction or discussion.

(4) The experiments are very thorough and the hypotheses thoroughly tested, but the sheer number of different comparisons and different control and experimental groups is somewhat overwhelming. It would be extremely helpful to provide results in table format, for example in listing the different groups and subgroups on p.6. Similarly, in the result section, there are (by my count) 12 paragraphs that start with the phrase "Compared with control..." This is acceptable, and much of this data is presented in histogram format in the figures. However the conclusions would be more clear if these experimental outcomes were displayed in table format.

(4) There are many figures and they are quite complex. The number of bars in each histogram require considerable effort to sort out what is experiment, what is control and what is being tested. Short statements in the figure legend summarizing the major point illustrated by that figure would be helpful, possibly in the first line, i.e. "treatment x increases and treatment y decreases the number of TH+ cells in 6-OHDA rats."

More specific comments:

(1) p.4 lines 16-26: the discussion of NLRPs effects in renal interstitial inflammation is not obviously relevant; the connection of this information to the point of this paper should be stated.

(2) abbreviations such as CARD and DA should be spelled out the first time they appear

(3) p.4 line 56: typographical error; "subjects and methods" should be a heading

(4) p.5 line 13: I would suggest using the word "discomfort" rather than "suffering."

(5) p.5 line 45: What is the negative control for the siRNA lentivirus?



(6) p.7: were the behavioral results (rotations) conducted by hand or were they automated? Were only complete rotations counted?

(7) Discussion first paragraph (p.14): This is just a list of background facts and information that seems to be presented in no particular order. If the authors feel it is important to make these points, they should be put into the appropriate point in the discussion. Possibly, this first paragraph could be removed entirely without harm to the discussion.